CLAIMS

1. A purified and isolated DNA sequence having protein production increasing activity characterized in that said DNA sequence comprises
   a) at least one bent DNA element,
   b) and at least one binding site for a DNA binding protein.

2. The purified and isolated DNA sequence of claim 1 characterized in that the bent DNA element contains at least 10% of dinucleotide TA, and/or at least 12% of dinucleotide AT on a stretch of 100 contiguous base pairs.

3. The purified and isolated DNA sequence of claim 2 characterized in that the bent DNA element contains at least 33% of dinucleotide TA, and/or at least 33% of dinucleotide AT on a stretch of 100 contiguous base pairs.

4. The purified and isolated DNA sequence of claims 1 to 2, characterized in that it comprises a MAR nucleotide sequence selected from the group comprising the sequences SEQ ID Nos 1 to 27, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

5. The purified and isolated DNA sequence of claims 1 to 2, characterized in that it comprises a cLysMAR element and/or fragment, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

6. The purified and isolated DNA sequence of claim 5, characterized in that said part thereof is a nucleotide sequence selected from the B, K and F regions.

7. The purified and isolated sequence of claims 1 to 6, characterized in that said DNA binding protein is a transcription factor.

8. The purified and isolated sequence of claim 7, characterized in that the transcription factor is selected from the group comprising the polyQpolyP domain proteins.

9. The purified and isolated sequence of claim 7, characterized in that the transcription factor is selected from the group comprising SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HFH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR, C/EBPgamma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3, TEF, VBP, XFD3, Brn2, COMP1, Evil, FOXP3, GATA4, HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFIIA and Vmw65 or a combination of two or more of these transcription factors.

10. A purified and isolated cLysMAR element and/or fragment having protein production increasing activity, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

11. The cLysMAR element and/or fragment of claim 10 consisting of at least one nucleotide sequence selected from the B, K and F regions.

12. A synthetic MAR sequence comprising natural MAR elements and/or fragments assembled between linker sequences.

13. The synthetic MAR sequence of claim 12, characterized in that the MAR comprises a cLysMAR element and/or fragment, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

14. The synthetic MAR sequence of claims 12 to 13, characterized in that the linker sequences are BglII-BamHI linker.

15. A method for identifying a MAR sequence using a Bioinformatic tool comprising the computing of values of one or more DNA sequence features corresponding to DNA bending, major groove depth and minor groove width potentials and melting temperature.

16. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 15, characterized in that said Bioinformatic tool contains algorithms, adapted to the use of profiles or weight-matrices, to compute values for one or more of said DNA sequence features corresponding to DNA bending, major groove depth and minor groove width potentials, and melting temperature.

17. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 16, characterized in that said profiles or weight-matrices are based on dinucleotide recognition.

18. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 17, characterized in that said Bioinformatic tool computes values for all of said DNA sequence features.

19. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 18, characterized in that said Bioinformatic tool is SMAR Scan®®.

20. The method for identifying a MAR sequence using a Bioinformatic tool according to claims 15-19, characterized in that the identification of one or more DNA sequence features further comprises a feature corresponding to one or more binding sites for DNA binding proteins.

21. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 20, characterized in that said DNA binding protein is a transcription factor.

22. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 21, characterized in that the transcription factor is selected from the group comprising polyQpolyP domain proteins or transcription factors.

23. The method for identifying a MAR sequence using a Bioinformatic tool according to claims 20 to 21, characterized in that the DNA binding protein is selected from the group comprising SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HFH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR, C/EBPgamma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3, TEF, VBP, XFD3, Brn2, COMP1, Evil, FOXP3, GATA4,

HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFIIA and Vmw65 or a combination of two or more of these transcription factors.

24. The method for identifying a MAR sequence using a Bioinformatic tool according to claims 15-23, characterized in that values for the identification of DNA bending are comprised between 3 to 5 °.

25. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 24, characterized in that values for the identification of DNA bending are comprised between 3.8 to 4.4 °.

26. The method for identifying a MAR sequence using a Bioinformatic tool according to claims 15-25 characterized in that values for the identification of the major groove depth are comprised between 8.9 to 9.3 Å and values for the identification of minor groove width are comprised between 5.2 to 5.8 Å.

27. The method for identifying a MAR sequence using a Bioinformatic tool according to claims 26, characterized in that values for the identification of major groove depth are comprised between 9.0 to 9.3 Å and values for the identification of minor groove width are comprised between 5.4 to 5.7 Å.

28. The method for identifying a MAR sequence using a Bioinformatic tool according to claims 15-27, characterized in that the melting temperature is comprised between 55 to 75 ° C.

29. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 28, characterized in that the melting temperature is comprised between 55 to 62 ° C.

30. The method for identifying a MAR sequence using a Bioinformatic tool of claims 15 to 29, characterized in that it further comprises at least one filter predicting DNA binding sites for DNA transcription factors.

31. The method for identifying a MAR sequence using a Bioinformatic tool according to claim 30, characterized in that the filter is applied before or after the Bioinformatic tool.

32. The method according to claims 30 to 31, characterized in that the filter detects clusters of DNA binding sites using profiles or weightmatrices.

33. The method according to claim 32, characterized in that the filter detects densities of clusters of DNA binding sites.

34. A method for identifying a MAR sequence characterized in that it comprises at least one filter detecting clusters of DNA binding sites using profiles or weightmatrices.

35. A purified and isolated MAR DNA sequence identifiable according to claims 15 to 33 or claim 34.

36. The purified and isolated MAR DNA sequence of claim 35, containing at least 10% of dinucleotide TA on a stretch of 100 contiguous base pairs.

37. The purified and isolated MAR DNA sequence of claim 36, containing at least 33% of dinucleotide TA on a stretch of 100 contiguous base pairs.

38. The purified and isolated MAR DNA sequence of claims 35 to 37, further containing at least 12% of dinucleotide AT on a stretch of 100 contiguous base pairs.

39. The purified and isolated MAR DNA sequence of claim 38, further containing at least 33% of dinucleotide AT on a stretch of 100 contiguous base pairs.

40. The purified and isolated MAR DNA sequence of any of claims 35 to 39, comprising a sequence selected from the sequences SEQ ID Nos 1 to 27, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

41. The purified and isolated DNA sequence of claim 40, comprising a sequence selected from the sequences SEQ ID Nos 24 to 27, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

42. The use of a purified and isolated DNA sequence comprising a first isolated matrix attachment region (MAR) nucleotide sequence which is a MAR nucleotide sequence selected from the group comprising:

- a purified and isolated DNA sequence of claims 1 to 9,
- a purified and isolated MAR DNA of claims 35 to 41,
- the sequences SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of claims 12 to 14,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants for increasing protein production activity in a eukaryotic host cell.

43. The use of the purified and isolated DNA sequence of claim 42, characterized in that said purified and isolated DNA sequence further comprises a promoter operably linked to a gene of interest.

44. The use of the purified and isolated DNA sequence of claims 42 or 43, characterized in that said purified and isolated DNA sequence further comprises at least a second isolated matrix attachment region (MAR) nucleotide sequence which is a MAR nucleotide sequence selected from the group comprising:

- a purified and isolated DNA sequence of claims 1 to 9,
- a purified and isolated MAR DNA of claims 35 to 41,
- the sequences SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of claims 12 to 14,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants for increasing protein production activity in a eukaryotic host cell.

45. The use of the purified and isolated DNA sequence of claim 44, characterized in that said first and at least second MAR sequences are located at both the 5' and the 3' ends of the sequence containing the promoter and the gene of interest.

46. The use of the purified and isolated DNA sequence of claim 44, characterized in that said first and or at least second MAR sequences are located on a sequence distinct from the one containing the promoter and the gene of interest.

47. The use of the purified and isolated DNA sequence of any of claims 42 to 46, characterized in that said purified and isolated DNA sequence is in the form of a linear DNA sequence as vector.

48. A method for transfecting a eukaryotic host cell, said method comprising
a) introducing into said eukaryotic host cell at least one purified DNA sequence comprising at least one DNA sequence of interest and/or at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements,
b) subjecting within a defined time said transfected eukaryotic host cell to at least one additional transfection step with at least one purified DNA sequence comprising at least one DNA sequence of interest and/or with at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements
c) selecting said transfected eukaryotic host cell.

49. The method of claim 48, characterized in that said DNA sequence of interest is a gene of interest coding for a protein operably linked to a promoter.

50. The method of claims 48 and 49, characterized in that the selected transfected eukaryotic host cells are high protein producer cells with a production rate of at least 10 pg per cell per day.

51. The method of claims 48-50, characterized in that the MAR nucleotide sequence is selected from the group comprising:
- a purified and isolated DNA sequence of claims 1 to 9,
- a purified and isolated MAR DNA of claims 35 to 41,
- the sequences SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of claims 12 to 14,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

52. The method of claims 48-50, characterized in that the MAR nucleotide is a purified and isolated sequence according to claims 1 to 9, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

53. The method of claims 48 to 52, characterized in that the defined time corresponds to intervals related to the cell division cycle.

54. The method of claim 53, characterized in that the defined time is the moment the host cell just has entered into a second cell division cycle.

55. A method for transfecting a eukaryotic host cell, said method comprising co-transfecting into said eukaryotic host cell at least one first purified and isolated DNA sequence comprising at least one DNA sequence of interest, and a second and isolated purified DNA comprising at least one MAR nucleotide selected from the group comprising:

- a purified and isolated DNA sequence of claims 1 to 9,
- a purified and isolated MAR DNA of claims 35 to 41,
- the sequences SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of claims 12 to 14,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants.

56. A process for the production of a protein wherein
a) a eukaryotic host cell transfected according to claims 48 to 54 or claim 55, is cultured in a culture medium under conditions suitable for expression of said protein and
b) said protein is recovered.

57. A eukaryotic host cell transfected according to any one of claims 48 to 54 or claim 55.

58. A cell transfection mixture or kit comprising at least one purified and isolated DNA sequence according to claims 1 to 9, 10 to 11, 12 to 14 or 35 to 41.

59. A transgenic organism characterized in that at least some of its cells have stably incorporated at least one DNA sequence of claims 1 to 9, 10 to 11, 12 to 14 or 35 to 41.

60. A transgenic organism characterized in that its genome has stably incorporated at least one DNA sequence of claims 1 to 9, 10 to 11, 12 to 14 or 35 to 41.

61. The transgenic organism of claims 59 and 60 characterized in that some of its cells have been transfected according to the method of claims 48 to 54 or claim 55.

62. A computer readable medium characterized in that it comprises computer-executable instructions for performing the method for identifying a MAR sequence of claims 15 to 33 and/or claim 34.